

mutant nucleic acid polymerase with canonical nucleoside triphosphates.

The present invention is also a method for determining the sequence of a nucleic acid molecule using a mutant RNA 5 polymerase.

The method comprises synthesizing a nucleic acid molecule, either *de novo* from a promoter, or by extending a primer annealed to the template molecule in four separate reactions. The four separate reactions each have all 4 10 rNTPs and a portion of a ddNTP, or have all 4 dNTPs and a portion of a ddNTP, or have 4 2'-fluorine-substituted NTPs and a portion of a ddNTP. Chain termination will occur and the products may be evaluated so that the sequence of the template molecule may be deduced. In one embodiment of this 15 method, the reactions which include a ddNTP occur in the same reaction mixture and are linked to a method for nucleic acid amplification, including, but not limited to, NASBA, 3SR, TMA, or other similar methods.

The present invention is also a partial ribo- 20 substitution method for determining the sequence of a nucleic acid molecule. This method comprises synthesizing a nucleic acid molecule, either *de novo* from a promoter or by extending a primer annealed to the template molecule in four separate reactions. The reactions each have, either four 25 dNTPs and a portion of an rNTP or four 2'-F-NTPs and a portion of an rNTP, or four different non-canonical nucleoside triphosphates, wherein these nucleoside 30 triphosphates have substituents different than a hydroxyl group at the 2' position of the ribose and which the mutant polymerase can use as substrates for synthesis in nucleic acids, and a portion of an rNTP. The reaction products are then cleaved at sites containing an incorporated rNTP by

using an alkaline solution or an RNase, and the cleaved nucleic acid fragments are separated according to size so that the sequence of the template molecule may be determined.

5       The present invention is also embodiments of a partial ribo-substitution method wherein the nucleic acid synthesis reactions of said method occur in the same reaction mixture and are also part of or linked to a method for nucleic acid amplification, including, but not limited to, NASBA, 3SR,  
10      TMA, or other similar methods.

In still other embodiments of the present invention, the products of either 1, 2, 3, or 4 of the di oxy-sequencing reactions or of the partial ribo-substitution sequencing reactions are performed or analyzed to determine  
15      the presence or absence of a particular nucleic acid, or its relatedness to another nucleic acid, or whether it contains a mutation compared to another nucleic acid.

The present invention is also a kit for performing any of the above-identified methods.

20      It is an object of the present invention to provide a mutant polymerase capable of altered discrimination between canonical and non-canonical nucleoside triphosphates.

It is an object of the present invention to provide an improved DNA sequencing method.

25      It is an object of the present invention to provide a method to detect the presence of a nucleic acid.

It is an object of the present invention to provide a method to detect the identity of a nucleic acid.

30      It is an object of the present invention to provide a method to detect mutations in a nucleic acid.

It is an object of the present invention to minimize the steps involved in amplifying and sequencing, detecting, identifying and detecting mutations in nucleic acids.

5 It is another object of the present invention to provide a method for synthesizing nucleic acid molecules with altered nuclease susceptibility.

10 It is another object of the present invention to provide a method for synthesizing nucleic acid molecules comprising at least one non-canonical nucleoside triphosphate.

Other objects, features and advantages of the present invention will become apparent after examination of the specification, claims and drawings.

#### Description of the Drawings

15 Fig. 1 diagrams the transcription products produced by Y639F and w.t. T7 RNAP in the presence of various combinations of rNTPs and dNTPs.

Fig. 2 shows the effect of dGTP substitution on transcription by the w.t. and Y639F polymerase.

20 Fig. 3 shows the effects of single-strandedness and sequence in the initially transcribed region on the activity of Y639F in reactions with 4 rNTPs or 4 dNTPs.

Fig. 4 shows transcription by Y639F and w.t. polymerase with dGTP or rGTP on poly(dI)•poly (dC).

25 Fig. 5 shows primed synthesis of DNA and RNA with Y639F and the w.t. polymerase.

Fig. 6 shows relative elongation rates of Y639F in "4 rNTP" and "3 rNTP + 1 dNTP" reactions.

30 Fig. 7 is a diagram of the mutagenesis strategy involved in creating a mutant SP6 RNA polymerase.